

## Case Report

# Recurrent retroperitoneal extra-GIST with rhabdomyosarcomatous and chondrosarcomatous differentiations: a rare case and literature review

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**Abstract:** Selective tyrosine kinase inhibitor (TKI) targeting KIT and PDGFRA is the frontline therapy for metastatic and unresectable GIST patients. Some initially responsive patients experience tumor progress because of secondary drug resistance, and some cases can develop heterogeneous differentiation. Here we report a rare case of recurrent retroperitoneal extra-GIST with rhabdomyosarcomatous and chondrosarcomatous differentiation with TKI therapy after surgical tumorectomy. Histology, immunohistochemistry, and mutational analysis were performed on primary and recurrent samples. The current case represents the first report of a recurrent retroperitoneal extra-GIST harboring mixed morphologic phenotypes of rhabdomyosarcoma and chondrosarcoma after TKI treatment. The dual differentiation can represent diagnostic pitfall.

**Keywords:** Extragastrointestinal stromal tumor, tyrosine kinase inhibitor, heterogeneous differentiation, rhabdomyosarcoma, chondrosarcoma

## Introduction

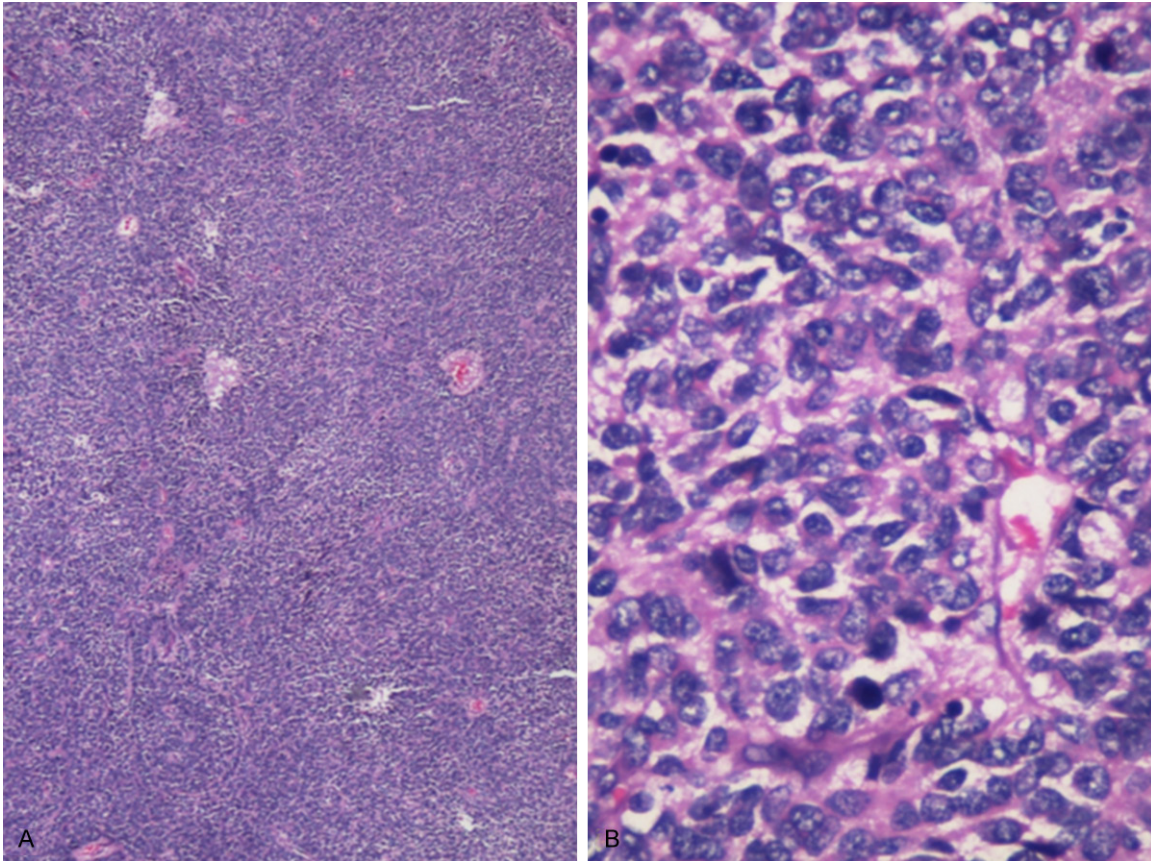
Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal (GI) tract [1]. Extragastrointestinal stromal tumors (extra-GISTs), arising from outside the GI tract but histologically and immunohistochemically resemble their GI counterparts, were also reported in recent years [2]. Most of GIST and extra-GIST have KIT or platelet-derived growth factor receptor-alpha (PDGFRA) mutations [3, 4]. In clinical practice, the availability of imatinib mesylate, a selective receptor tyrosine kinase inhibitor (TKI) that targets the KIT and PDGFRA, has revolutionized GIST treatment and is now the first-line standard treatment for patients with surgically unresectable or metastatic disease [5, 6]. After an initial benefit from TKI, the vast majority of patients eventually develop disease progression or secondary resistance mainly because of acquired mutations in KIT or PDGFRA [7]. Most of resistant cases retain typical morphology of GIST, but some cases can develop heterogeneous differentiation [8, 9].

Here, we present a highly rare case of extra-GIST with rhabdomyosarcomatous and chondrosarcomatous differentiation after TKI treatment. To our knowledge, this is the first report of dual heterologous differentiations developing in retroperitoneal extra-GIST after TKI treatment. The study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Written informed consent was obtained from the patient.

## Case report

A 57-year-old man with no history of relevant disease was diagnosed with abdominal multinodular mass on clinical evaluation for abdominal discomfort. Ultrasonography and computed tomography scan confirmed the presence of retroperitoneal multi-nodules with 1.2 cm in its greatest dimension. The results of upper GI endoscopy and colonoscopy revealed no pathological findings. The pathology diagnosis was malignant retroperitoneal extra-GIST. Subsequently routine treatment with the tyrosine

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**Figure 1.** Primary tumor specimen of retroperitoneal extra-GIST shows spindle-shaped cells of a high dense cellularity, hyperchromasia, and nuclear atypia, and a high mitotic activity (A. ×100; B. ×400).

kinase inhibitor (TKI) imatinib mesylate after surgical tumorectomy was performed. Fifteen months later, the patient was diagnosed with multifocal retroperitoneal recurrence. At the time of recurrence, the primary and recurrent tumors were further characterized for genetic and molecular abnormalities, and the recurrent one finally was diagnosed as recurrent retroperitoneal extra-GIST with heterogeneous rhabdomyosarcomatous and chondrosarcomatous differentiations. The patient showed continued tumor progression and died of infection and multisystem organ failure 42 months after initial diagnosis.

### Materials and methods

Specimens from primary and recurrent tumors were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) by use of routine procedures.

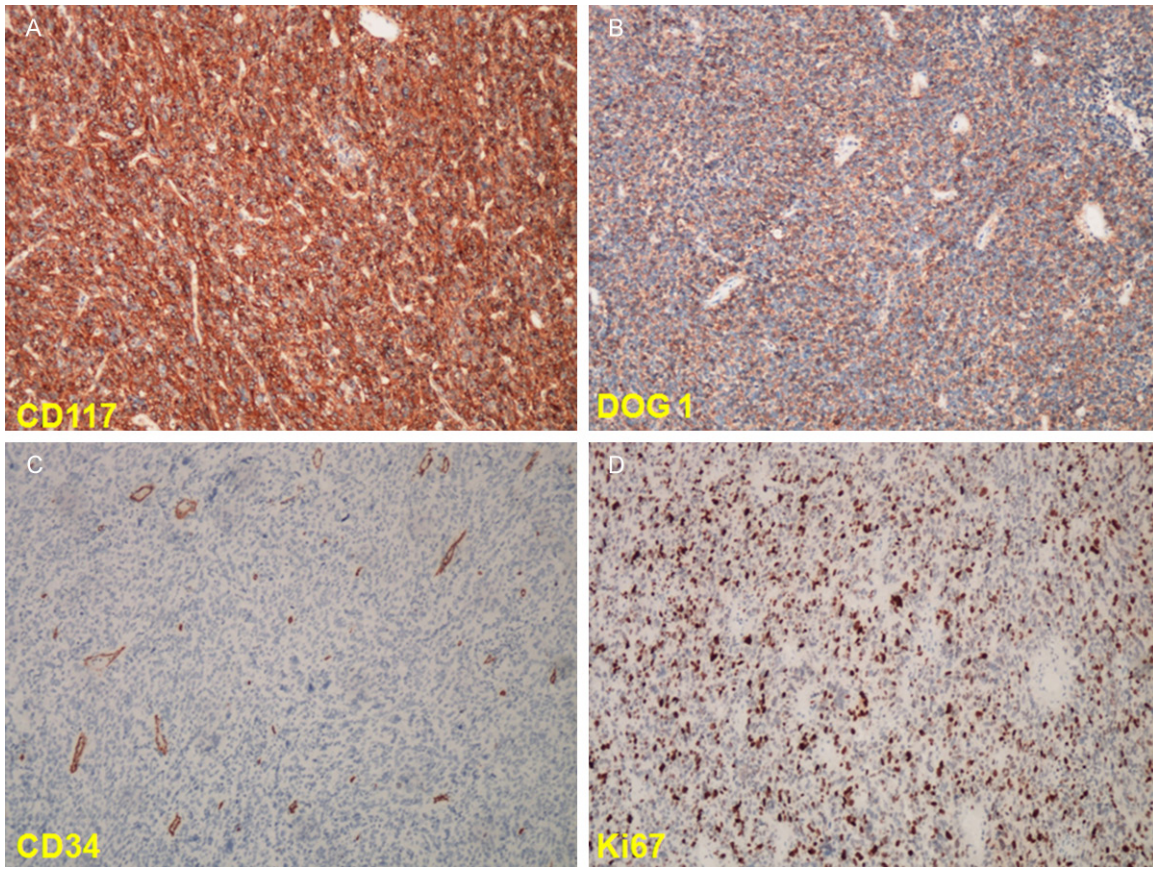
Immunostaining was performed by an enhancement method based on repetitive microwave

heating of slides that were placed into 0.01 M citrate buffer at pH 6.0. Binding of primary antibodies was visualized with an Envision two-step method. A panel of antibodies was used, either monoclonal or prediluted. They were specific for CD117 (1:40; Dako), CD34 (1:20; Dako), DOG1 (1:50; Novocastra), S100 (1:1000; Dako),  $\alpha$ -smooth muscle actin (1:100; Dako), desmin (1:100; Dako), Myogenin (1:100; Dako), MDM2 (1:100; Dako), Ki67 (prediluted; Dako). Diaminobenzidine was used as chromogen. Nuclei were stained with Mayer's hematoxylin. Appropriate positive controls were included.

Mutational analysis was performed on primary and recurrent samples in the areas showing classic GIST morphology and areas with chondrosarcomatous and rhabdomyosarcomatous differentiation. Selected paraffin-embedded tissue was deparaffinized by serial extraction with xylene and ethanol and allowed to air dry. DNA was extracted using the Qiagen minikit (Qiagen, Valencia, CA) in accordance with the



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**Figure 2.** Primary tumor of retroperitoneal extra-GIST was confirmed by immunopositivity for CD117 and DOG1 with high Ki-67 labeling index, but immunonegativity for CD34.

manufacturer's recommendations. Mutational analysis were performed on the extracted genomic DNA using a combination of polymerase chain reaction amplification, denaturing high-performance liquid chromatography screening, and automated sequencing. All samples were evaluated for mutations in KIT exons 9, 11, 13, 14, 17, and 18 and PDGFRA exons 12, 14 and 18.

### Results

#### *Pathology examination*

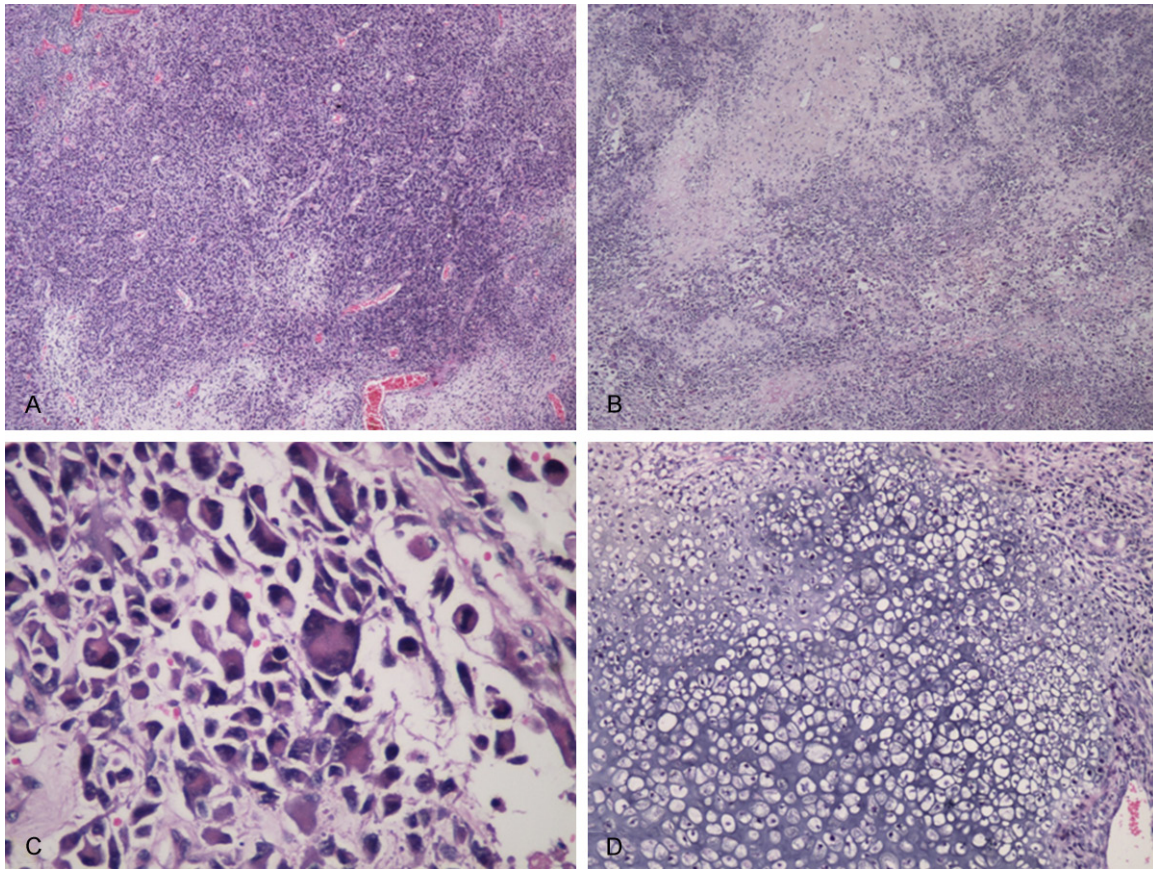
Grossly, pretreatment tumor specimens showed multi-nodular masses with a range from 4 cm to 12 cm in their dimensions. The cut surface was grey-yellow with necrosis, hemorrhage. The recurrent tumor specimens showed similar appearances macroscopically but more nodules and bigger in their dimensions (18 cm in the greatest dimension) diffused in retroperitoneal area.

Microscopically, the primary tumor was composed of spindle-shaped cells of a high dense cellularity, hyperchromasia, and nuclear atypia, and a mitotic activity of 5/50 HPF with focally hemorrhage and necrosis (**Figure 1A, 1B**).

In the recurrence, morphologic features in most area of the tumor were similar to the primary one with dense spindle cells (**Figure 3A**). Focally, chondroid matrix with variable cellularity, and chondrocytes containing nuclei with open chromatin patterns, nucleoli, and mitoses within the matrix form clusters which resembling well-differentiated chondrosarcoma could be observed (**Figure 3B, 3D**); as well as large, polygonal tumor cells with vesicular nuclei, prominent nucleoli, and brightly eosinophilic cytoplasm resembling pleomorphic rhabdomyosarcoma (**Figure 3B, 3C**). Tumor areas showing rhabdomyosarcomatous and chondrosarcomatous differentiations were present next to areas with classic extra-GIST morphology.



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**Figure 3.** Recurrent tumor showing classic morphology of extra-GIST (A.  $\times 100$ ) and heterogeneous rhabdomyosarcomatous and chondrosarcomatous differentiation (B.  $\times 100$ ; C, D.  $\times 200$ ).

### *Immunohistochemistry*

The primary tumor tissue showed strong positivity with CD117 (**Figure 2A**) and discovered on GIST-1 (DOG1) (**Figure 2B**), whereas immunoreactivity with CD34 (**Figure 2C**), smooth muscle actin (SMA) and desmin was negative. In the recurrent specimens, the area showing classic GIST morphology expressed CD117 (**Figure 4A**) and DOG1 as well. The rhabdomyosarcomatous component present demonstrated consistently strong positivity for desmin (**Figure 4B**) and myogenin (**Figure 4C**). S-100 (**Figure 4D**) expression was observed in the chondrosarcomatous component. In both pretreatment and posttreatment specimens, MDM2, AE1/AE3, epithelial membrane antigen (EMA) and CK8/18 were negative, and Ki67 label index was relatively high (**Figure 2D**).

### *Mutation analysis*

In both primary and recurrent specimens from classic extra-GIST area, a KIT exon 11 point

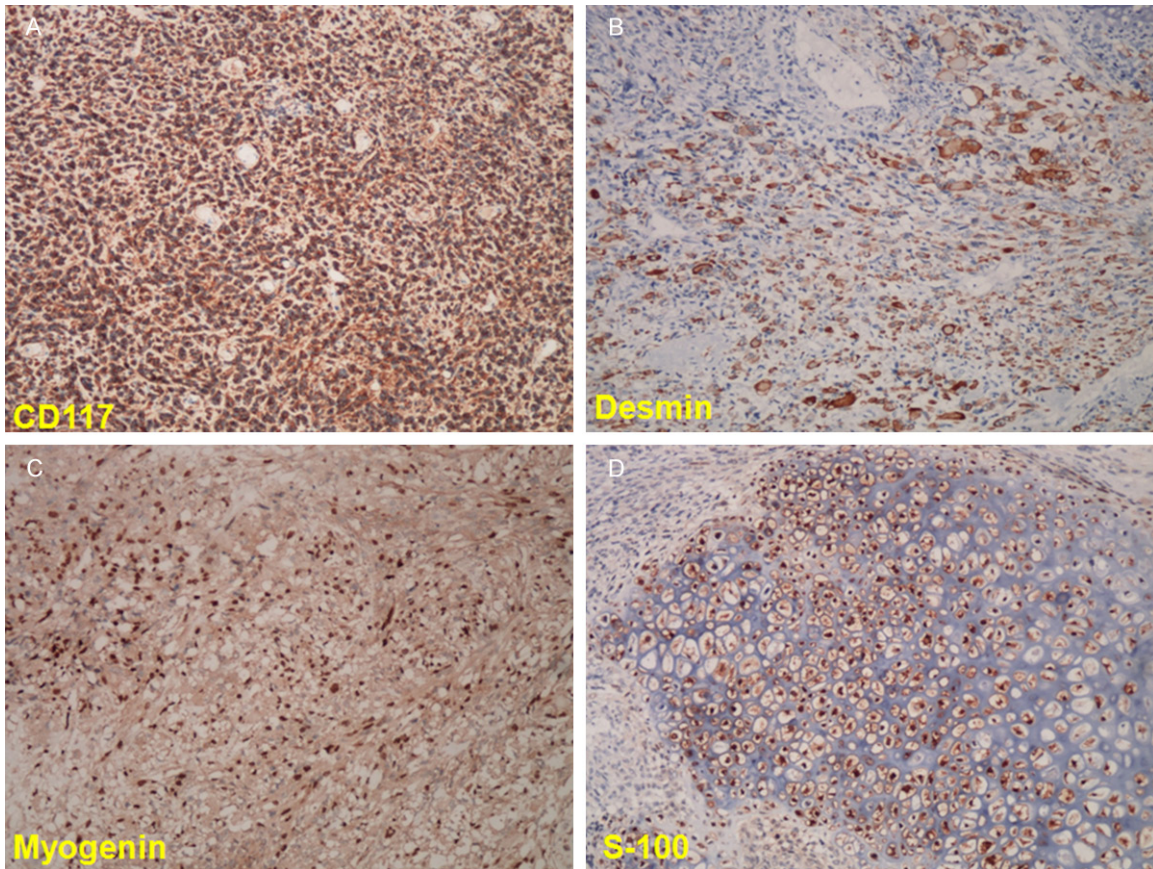
mutation W557R was observed. All selected specimens with rhabdomyosarcomatous and chondrosarcomatous differentiation from recurrent tumor retained the primary KIT mutation demonstrated in samples with classic extra-GIST morphology, but did not show secondary KIT/PDGFR $\alpha$  resistance mutations.

### **Discussion**

Gastrointestinal stromal tumor (GIST) is currently considered a distinct clinicopathologic and molecular entity since Hirota et al reported that the tumor can harbor activating mutations in the KIT receptor tyrosine kinase (RTK) gene and commonly express KIT protein (CD117) by immunohistochemistry [3]. Discovered on GIST-1 (DOG1) subsequently were confirmed as special markers of GIST [10]. Extra-GIST, although uncommon reported, shared the same histopathologic and molecular features with GIST [2, 11]. In clinical practice, therapeutic inhibition of KIT/PDGFR $\alpha$  kinase activity by imatinib mesylate has emerged as the effective treatment



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**Figure 4.** Recurrent tumor with dual transdifferentiation was confirmed by immunopositivity for CD117 in classic GIST area, desmin, myogenin in rhabdomyosarcomatous area and S-100 in chondrosarcomatous area.

option in patients with primary unresectable and metastatic GIST. However, most patients subsequently experience disease progress because of the acquired resistance [12, 13]. Unusual morphological and immunohistochemical features are found in GISTs after TKI treatment, and heterogeneous differentiation including epithelioid sarcoma, rhabdomyosarcoma and smooth muscle tumor and so on were reported [8, 9, 14] (**Table 1**). As we known by checking previous references, all the reported cases with heterogeneous differentiation after TKI treatment had single transdifferentiation pattern, and there was no dual differentiation report before. The current case represents the first report of a recurrent retroperitoneal extra-GIST harboring mixed morphologic phenotypes of rhabdomyosarcoma and chondrosarcoma after TKI treatment.

Although the specific mechanism for the phenotypic changes of GIST with TKI treatment has not been identified, some investigators believed

that KIT alterations may lead to the phenotypic expression of more highly differentiated mesenchymal elements [15]. However, there are conflicting results from others that there were morphologic changes without secondary KIT mutation after TKI treatment [7]. It is also possible that differential activation of downstream effectors of the KIT pathway may influence cellular differentiation to express more mature mesenchymal elements. Alternatively, a secondary KIT/PDGFR $\alpha$ -independent mutation may be responsible for the observed phenotypic changes.

Differential diagnostic difficulties are more often encountered with Dedifferentiated liposarcoma and malignant mesenchymoma in present case.

Dedifferentiated liposarcoma (DLPS) is one of the most frequent malignant soft tissue tumors of the retroperitoneum. In some cases, the differentiated component is a heterologous sar-

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**Table 1.** Summary of reported cases of heterogeneous differentiation of GIST and extra-GIST

Case (reference)	Anatomic location	Morphology	Year
1 (Torihashi S, et al)	small intestine	smooth muscle	1999
2 (Leung KM, et al)	stomach	smooth muscle	2002
3 (Richmond JA, et al)	stomach	rhabdoid phenotype	2004
4 (Furihata M, et al)	gallbladder	rhabdomyomatous phenotype	2005
5 (Pauwels P, et al)	stomach	Epithelioid phenotype	2005
6 (Bickenbach K, et al)	stomach	epithelioid differentiation and bone and cartilage formation	2007
7 (Bumming P, et al)	stomach	neuroendocrine phenotype	2007
8 (Kocer NE, et al)	Ampulla of Vater	osteoclast-like giant cells	2007
9 (Liegl B, et al)	Stomach and small bowel	rhabdomyoblastic differentiation	2009
10 (Rahimi K, et al)	appendix	autonomic nerve differentiation	2009
11 (Vassos N, et al)	stomach	epithelioid sarcomatous phenotype	2011

coma subtype including leiomyosarcoma, rhabdomyosarcoma, and osteosarcoma. DLPS is the first exclusion that should be considered in retroperitoneum before other decision will be made. MDM2 is a sensitive and specific marker of DLPS by immunohistochemistry and correlates with gene amplification. We confirmed that there was no positive staining for MDM2 immunohistochemically or gene amplification, but positive for CD117 and DOG1 in primary and current case. Leiomyosarcoma, and other rare subtypes including fibrosarcoma and malignant peripheral nerve sheath tumors can also be ruled out based on morphologic and immunohistochemical features.

Another extremely rare sarcoma, so-called malignant mesenchymoma should also be excluded for the diagnosis. The tumor is defined as a malignant soft tissue tumor that consists of two or more distinctly different mesenchymal components, including osteosarcoma, chondrosarcoma, leiomyosarcoma, and rhabdomyosarcoma, in addition to fibrosarcomatous elements. Malignant mesenchymoma can occur at multiple locations including the retroperitoneum, soft tissue of the lower extremities, heart and so on. We could exclude the malignant mesenchymoma from current case because of immunopositivity for DOG1 and CD117 combined with the history of extra-GIST.

In summary, we present here a very rare case of recurrent extra-GIST with unique dual morphologic features of chondrosarcoma and rhabdomyosarcoma differentiation after TKI treatment. This finding can represent a diagnostic pitfall. Awareness of this unusual phenotypic change could prevent diagnostic confusion and

false interpretation as other sarcomas by the pathologists. Further exploration of the alternative pathways involved in the case would shed some light on their pathogenetic mechanisms, with the potential of targeting these alternative resistance mechanisms.

### Disclosure of conflict of interest

None.

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### References

- [1] Kindblom LG, Remotti HE, Aldenborg F and Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. *Am J Pathol* 1998; 152: 1259-1269.
- [2] Reith JD, Goldblum JR, Lyles RH and Weiss SW. Extragastrointestinal (soft tissue) stromal tumors: an analysis of 48 cases with emphasis on histologic predictors of outcome. *Mod Pathol* 2000; 13: 577-585.
- [3] Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y and Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998; 279: 577-580.
- [4] Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD and Fletcher JA. PDGFRA activating mutations

## Recurrent extra-GIST with two differentiations

- in gastrointestinal stromal tumors. *Science* 2003; 299: 708-710.
- [5] Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B and Demetri GD. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 2001; 344: 1052-1056.
- [6] Gronchi A, Blay JY and Trent JC. The role of high-dose imatinib in the management of patients with gastrointestinal stromal tumor. *Cancer* 2010; 116: 1847-1858.
- [7] Heinrich MC, Maki RG, Corless CL, Antonescu CR, Harlow A, Griffith D, Town A, McKinley A, Ou WB, Fletcher JA, Fletcher CD, Huang X, Cohen DP, Baum CM and Demetri GD. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol* 2008; 26: 5352-5359.
- [8] Liegl B, Hornick JL, Antonescu CR, Corless CL and Fletcher CD. Rhabdomyosarcomatous differentiation in gastrointestinal stromal tumors after tyrosine kinase inhibitor therapy: a novel form of tumor progression. *Am J Surg Pathol* 2009; 33: 218-226.
- [9] Vassos N, Agaimy A, Schlabrakowski A, Hohenberger W, Schneider-Stock R and Croner RS. An unusual and potentially misleading phenotypic change in a primary gastrointestinal stromal tumour (GIST) under imatinib mesylate therapy. *Virchows Arch* 2011; 458: 363-369.
- [10] West RB, Corless CL, Chen X, Rubin BP, Subramanian S, Montgomery K, Zhu S, Ball CA, Nielsen TO, Patel R, Goldblum JR, Brown PO, Heinrich MC and van de Rijn M. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. *Am J Pathol* 2004; 165: 107-113.
- [11] Kim KH, Nelson SD, Kim DH, Choi KU, Kim SJ, Min KW, Jang KS, Paik SS, Oh YH, Chae SW, Sohn JH, Kim HJ, Cho YK, Kim BI, Park DI, Sohn CI, Oh S, Choi SH, Choi YJ, Woo HY, Park YL, Park SJ, Lee SH, Ryu S, Do SI, Kang G, Kim K, Cho YH and Pyo JS. Diagnostic relevance of overexpressions of PKC-theta and DOG-1 and KIT/PDGFR gene mutations in extragastrointestinal stromal tumors: a Korean six-centers study of 28 cases. *Anticancer Res* 2012; 32: 923-937.
- [12] Wardelmann E, Buttner R, Merkelbach-Bruse S and Schildhaus HU. Mutation analysis of gastrointestinal stromal tumors: increasing significance for risk assessment and effective targeted therapy. *Virchows Arch* 2007; 451: 743-749.
- [13] Antonescu CR. Targeted therapy of cancer: new roles for pathologists in identifying GISTs and other sarcomas. *Mod Pathol* 2008; 21 Suppl 2: S31-36.
- [14] Torihashi S, Nishi K, Tokutomi Y, Nishi T, Ward S and Sanders KM. Blockade of kit signaling induces transdifferentiation of interstitial cells of cajal to a smooth muscle phenotype. *Gastroenterology* 1999; 117: 140-148.
- [15] Bickenbach K, Wilcox R, Veerapong J, Kindler HL, Posner MC, Noffsinger A and Roggin KK. A review of resistance patterns and phenotypic changes in gastrointestinal stromal tumors following imatinib mesylate therapy. *J Gastrointest Surg* 2007; 11: 758-766.